Enzymatic Chlorophyll Degradation in Wheat Near-Isogenic Lines Elicited by Cereal Aphid (Homoptera: Aphididae) Feeding

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J. Econ. Entomol. 97(2): 661–667 (2004)

ABSTRACT Chlorophyll degradation enzyme (i.e., chlorophyllase, Mg-dechelatase, and chlorophyll oxidase) activities of aphid-infested and uninfested 'Tugela' and Tugela near-isogenic wheat lines (i.e., Tugela-*Dn1*, Tugela-*Dn*2, and Tugela-*Dn*5) were assayed. Chlorophyllase activity was higher in bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae),-infested wheat lines compared with Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae)]-infested and uninfested plants. Mg-dechelatase activity was higher in *D. noxia*-infested wheat lines than in *R. padi*-infested and uninfested plants. Also, Mg-dechelatase activity was lower in Tugela wheat infested with *D. noxia* than in Tugela near-isogenic lines with *Dn* genes. Based on the in vitro assays of chlorophyll degradation enzyme (i.e., chlorophyllase and Mg-dechelatase) activities, we proposed that the chlorotic symptoms observed on *D. noxia*-infested Tugela wheat were most likely to be elicited by unbalanced chlorophyll biosynthesis and degradation.

KEY WORDS *Diuraphis noxia*, *Rhopalosiphum padi*, chlorophyllase, Mg-dechelatase, chlorophyll oxidase

THE RUSSIAN WHEAT APHID, *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae), is a serious pest of cereal crops worldwide, except in Australia. This aphid species has a wide range of cereal grains and grasses as host plants. Chlorosis is the most obvious plant injury symptom on cereal plants after *D. noxia* feeding and is indicative of chlorophyll loss (Burd and Elliott 1996, Rafi et al. 1996, Heng-Moss et al. 2003).

Enzymatic chlorophyll degradation in higher plants has been studied mainly on senescent barley, *Hordeum vulgare* L., leaves and cotyledons of oilseed rape, *Brassica napus* L. (Matile et al. 1996, 1999; Hörtensteiner 1999; Kräutler and Matile 1999). Other angiosperm species such as citrus, soybean, spinach, cucumber, and tobacco and algal species such as *Chlorella protothecoides* have also been studied in terms of chlorophyll degradation. Two chlorophyll degradation pathways, the pheophorbide *a* and oxidative bleaching pathways, have been described previously (Janave 1997, Matile et al. 1999, Dangl et al. 2000, Takamiya et al. 2000) (Fig. 1). In the pheophorbide *a* pathway, chlorophyllase catalyzes dephytylation and transforms chlorophyll into chlorophyllide. Mg-dechelatase then removes magnesium from the tetrapyrrole macrocycle and yields pheophorbide *a*. There are additional modifications of the tetrapyrrole ring such as oxygenolytic cleavage of the tetrapyrrole ring by pheophorbide *a* oxygenase and conversion of the cleavage product to colorless fluorescent compounds by red chlorophyll catabolite reductase and, ultimately, to nonfluorescent compounds (Ginsburg and Matile 1993, Hörtensteiner et al. 1995, Mühlecker and Kräutler 1996, Hörtensteiner et al. 1998, Malkin and Niyogi 2000). Janave (1997) suggested the oxidative bleaching pathway when examining the chlorophyll degradation initiated by enzymes extracted from Cavendish bananas, *Musa cavendishi* L., and found chlorophyll oxidase activity was inhibited under anoxygenic conditions or by adding ascorbate (Fig. 1). However, in the late stage of chlorophyll biosynthesis, protoporphyrin IX is metallated into chlorophyllide *a* by insertion of Mg from Mg-chelatase, which is a two-step reaction activated by ATP (Walker and Wenstein 1994, Blankenship 2002). In the following step, attachment of phytol chain to chlorophyllide *a* is catalyzed by chlorophyll synthase (Blankenship 2002).

Although chlorophyll degradation in plants has been extensively studied in terms of normal progressive senescence in many higher plants, little is known about chlorosis formation in plants elicited by herbivore feeding, especially in wheat, *Triticum aestivum* L. Because *D. noxia* elicits plant chlorosis, it is important to monitor chlorophyll degradation enzyme activities in herbivore-

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0022-0493/04/0661–0667$04.00/0

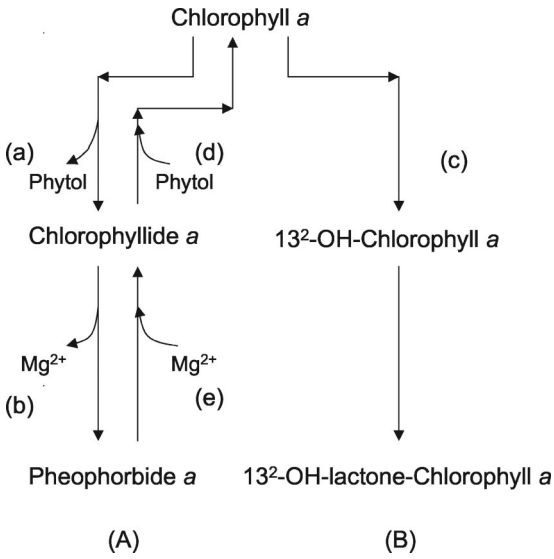

Fig. 1. Chlorophyll degradation (downstream) and biosynthesis (upstream) in wheat. (A) Chlorophyll pheophorbide *a* pathway described by Takamiya et al. (2000). (B) Chlorophyll oxidative bleaching pathway described by Janave (1997); (a) chlorophyllase; (b) magnesium-dechelatase; (c) chlorophyll oxidase; (d) chlorophyll synthase; and (e) magnesium-chelatase.

infested plants and to determine whether chlorosis formation is similar to chlorophyll degradation found in naturally senescing plants. Ni et al. (2001, 2002) in their enzymatic analysis of *D. noxia*-infested 'Arapahoe' wheat, did not detect either chlorophyllase or oxidative bleaching activity described by Matile et al. (1999) and Janave (1997) in natural senescing plants. They proposed that herbivore-elicited chlorophyll loss was different from the process observed in naturally senescing plants and suggested aphid-elicited chlorosis is related to the increase of Mg-dechelatase activity and other plant physiological or biochemical changes.

The present research is a continuation of research previously reported by Ni et al. (2001, 2002) to understand the biochemical mechanisms of aphid-elicited chlorosis by using 'Tugela' and *D. noxia*-resistant wheat isolines (i.e., Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*). The main objectives were to determine changes in chlorophyll degradation enzyme (i.e., chlorophyllase, Mg-dechelatase, and chlorophyll oxidase) activities elicited by aphid feeding in wheat lines with varying *D. noxia* resistance and to assess the correlation of *Dn* genes with chlorosis formation.

Materials and Methods

Plants and Insects. *D. noxia*, a chlorosis-eliciting species, and bird cherry-oat aphid, *Rhopalosiphum padi* (L.), a nonchlorosis-eliciting species, were used in the experiment. Both aphids were from colonies established from field collections. The *D. noxia* colony was established from aphids collected near Scottsbluff, NE, in 1994, whereas the *R. padi* colony was established from aphids collected near Lincoln, NE, in 1996 (Ni et al. 2001). Aphids were maintained on 'Stephens' wheat (*D. noxia*-susceptible) in Plexiglas cages (30 by 15 by 15 cm) in two separate Conviron growth chambers (Controlled Environments Ltd., Winnipeg, MB, Canada) at 21°C, with a photoperiod of 16:8 (L: D) h and 40–50% RH.

Wheat lines used in the experiment varied in their susceptibility to *D. noxia.* The 'Tugela' wheat was *D. noxia* susceptible, whereas the near-isogenic lines varied in *D. noxia* resistance (Tugela-*Dn1*, antibiosis; Tugela-*Dn2*, tolerance; and Tugela-*Dn5*, antixenosis and antibiosis) (du Toit 1987, 1989). Seeds were planted at the rate of two plants per Conetainer (3.81 cm in diameter by 21 cm in depth) (Stuewe and Sons, Inc., Corvallis, OR). Conetainers were filled with Sunshine soil mix No.1 (SunGro Horticulture, Bellevue, WA) and placed in Conetainers racks (61 by 30 by 18 cm), leaving a space between Conetainers to provide adequate light. Plants were watered uniformly from the bottom by placing a Conetainer rack over a plastic tray (54 by 28 by 6 cm) filled with water. The Tugela lines were maintained in a growth chamber at 21°C with a photoperiod of 16:8 (L: D) h and 40–50% RH for 13 d until they were infested with aphids.

Aphid Infestation and Plant Sample Collection. Aphids were preconditioned on 'Stephens' wheat for one generation following procedures of Schotzko and Smith (1991). Apterous adults of *D. noxia* and *R. padi* ($n = 10$) were placed on 'Stephens' wheat plants at the three-leaf stage (Zadoks stage 13) (Zadoks et al. 1974) and then caged with polyethylene tube-cages (30 cm in length by 4 cm in diameter). After 3 d of infestation, adults were removed and nymphs were maintained on the plants for 7 or 8 d before infestation. Thus, the variation of aphid age was within 3 d and provided relatively age-specific aphids for our experiment.

There were three levels of aphid infestation on plants: 0 aphid, 10 apterous *R. padi* adults, and 10 apterous *D. noxia* adults. The experiment was initiated when plants were at the three-leaf stage. After being infested with aphids, all plants were caged using polyethylene tube-cages (30 cm in length by 4 cm in diameter) and maintained in Conviron growth chambers under the conditions described previously. Aphids and wheat plants were collected and weighed on the 3rd, 6th, 9th, and 12th d after initial aphid infestation. On each sampling date, three Conetainers (two plants per Conetainer) of each genotype (i.e., Tugela, Tugela-*Dn1*, Tugela-*Dn2* and Tugela-*Dn5*) under each aphid treatment (i.e., control, *D. noxia*-infested, and *R. padi*-infested) were randomly collected. The two wheat plants from each Conetainer were excised, aphids were removed, and aphids and plants were weighed. Because the wheat leaf blades of each plant (≈0.5 g on day 3 and 1.5 g on day 12) are relatively small, leaves of three samplings within a treatment were combined and processed as one sample (≈3 g on day 3 and 10 g on day 12).

Enzyme Extraction of Plant Samples. The enzyme extraction from wheat leaf samples was conducted according to methods reported by Mihailoviæ et al.

(1997) and modified based on the reports by Ellsworth (1971) and Janave (1997). Wheat leaves (3.0–10.0 g) were ground with liquid nitrogen in a mortar and pestle. Chilled extraction buffer (20 ml) containing 0.1 M potassium phosphate buffer (pH 6.2), 1% NaCl, 1% Triton X-100, and 1% polyvinylpyrrolidone was used for all plant samples. Plant homogenates were filtered through one layer of Miracloth. Chlorophylls were separated and removed once using one volume of *n*-butanol (Aldrich Chemical, Milwaukee, WI) and centrifuged at 3,000 × *g* for 3 min.

The protein in the lower (aqueous) layer was collected and precipitated with 3 volumes of cold (4°C) acetone. The mixture was swirled briefly and allowed to stand in ice for 10 min. The samples were then centrifuged at 10,000 × *g* for 10 min. The precipitate was resuspended in 1 ml of 0.1 M potassium phosphate buffer (pH 7.0) and held at 4°C for 2 h before initiation of enzyme assays. Only fresh enzyme samples were used for the assays.

Preparation of Enzyme Assay Substrates. Quantified substrates were prepared to assay chlorophyll degradation enzyme activities from wheat lines. Fresh spinach leaves were used for chlorophyll extraction according to Janave (1997). After the spinach leaves were ground in chilled acetone, the extracts were filtered through Whatman No. 2 filter paper and then centrifuged at 6,000 × *g* for 10 min to remove insoluble plant tissues. The supernatant was purified twice by dioxane precipitation (Iriyama et al. 1974, Janave 1997). The ratio of dioxane: acetone was 1:7 (vol:vol). The distilled water was then added dropwise while swirling the mixture until a precipitate was formed. The precipitated chlorophylls were centrifuged at 3,000 × *g* for 3 min and resuspended in absolute acetone. The concentration of chlorophyll *a* was determined by diluting the original chlorophyll solution with 80% acetone, and concentration was calculated according to the formula described by Bertrand and Schoefs (1997) by using absorbance of 646 and 663 nm. The chlorophyll solution was placed in the microcentrifuge tubes wrapped with aluminum foil and stored in dark at −20°C. Prepared chlorophyll solution was used as the substrate for total chlorophyll degradation and chlorophyllase activity assays.

Chlorophyllin was prepared from chlorophyll by Molisch conversion as described by Vicentini et al. (1995). Spinach leaves (≈10 g) were ground in 80% acetone. The chlorophyll was partitioned in petroleum ether phase in a ratio of 1:1 (vol:vol). The petroleum ether phase was washed twice with distilled water. One hundred microliters of 30% KOH in methanol was added to 12 ml of chlorophyll solution in petroleum ether. The precipitated chlorophyllin was centrifuged at 3,000 × *g* for 5 min and dissolved in 10 ml of distilled water. The pH of the chlorophyllin solution was adjusted to pH 9 by adding tricine. Chlorophyllin solution is stable at pH 9 when stored in the dark at −20°C. Chlorophyllin was used as the substrate for the Mg-dechelatase assay.

Protein Determination. Protein concentration of all leaf samples was determined according to the dye-binding assay (Bollag and Edelstein 1991) by using bovine serum albumin (Sigma, St. Louis, MO) as a standard. The enzyme extracts used in protein determination were treated with butanol (to remove pigments) and acetone (to concentrate protein) for assays of chlorophyll catabolic enzyme activities.

Total Chlorophyll Degradation Assay. The disappearance of chlorophyll *a* was measured according to the method used by Janave (1997). The reaction mixture (1.0 ml) contained 0.36 ml of 0.1 M potassium phosphate buffer (pH 7.0), 0.288 ml of acetone (to make 30% in final reaction mixture), 12 μl of chlorophyll in acetone (to make 10 μM of chlorophyll *a* in final reaction), and 0.34 ml of enzyme extract. The control mixture did not contain enzyme extract (0.7 ml of potassium phosphate buffer instead). The mixtures were incubated at 30°C in a water bath under dark condition for 30 min. The reaction was stopped by adding 0.1 ml of 1 N NaOH and followed by 3 ml of acetone/hexane mixture [one-half (vol:vol)]. The reaction mixture was vortexed vigorously until emulsion formation, allowed to stand for 10 min, and centrifuged at 3,000 × *g* for 5 min. The absorbance of the hexane layer at 663 nm was recorded. Chlorophyll *a* concentration was determined by using the specific absorption coefficient of 94.5 M^{-1} cm^{-1}. Activity was expressed by micromoles of chlorophyll *a* degraded per 30 min per gram of fresh leaf weight. Chlorophyll degradation in this protocol was the result of both chlorophyllase and chlorophyll oxidase (also known as chlorophyll oxidative bleaching) activities (Janave 1997).

Chlorophyllase Activity. Chlorophyllase activity was determined using a modified procedure based on the reports on Cavendish banana (Janave 1997) and rye, *Secale cereale* L., seedlings (Tanaka et al. 1982). The reaction mixture containing 0.35 ml of 0.1 M potassium phosphate buffer (pH 7.0), 0.288 ml of acetone (to make 30% in final concentration), 0.01 ml of 0.1 M ascorbate (to inhibit the oxidative bleaching pathway or chlorophyllase oxidase activities), and 12 μl of chlorophyll (for final concentration of 10 mM). The reaction was initiated by adding 0.34 ml enzyme extract. After 30 min at 30°C, 0.1 ml of 1 N NaOH was added to stop the reaction. Then, 3 ml of acetone/*n*-hexane [one-half (vol:vol)] were added to the reaction mixture. The mixture was vigorously shaken to allow the chlorophyllide formed by the enzymatic reaction to be partitioned into the lower aqueous layer. The mixture was centrifuged at 3,000 × *g* for 5 min. The enzyme activity was determined according to the decrease of chlorophyll *a* by using the absorbance changes at 663 nm. The reduction of chlorophyll *a* only indicated chlorophyllase activity.

Mg-Dechelatase Activity. The dechelation of magnesium from chlorophyllin (or chlorophyllide) to pheophorbide was determined by monitoring the change in absorbance with time at 686 nm according to Vicentini et al. (1995) and Janave (1997). The assay mixture was comprised of 800 μl of 50 mM Tris-tricine (pH 8.0), 95 μl of chlorophyllin (A_{686} nm = 0.1), 100 μl of 1% Triton X-100, and 5 μl of enzyme extract. The

control mixture did not contain enzyme extract. The reaction was carried out at 25°C and activity expressed as ΔA_{686} per minute per gram of leaf. The decrease of substrate chlorophyllide and the increase of pheophorbide in 2 min were monitored on a spectrophotometer (model Genesys 5, Spectronic Instruments, Rochester, NY) based on the protocol described by Janave (1997).

Chlorophyll Oxidase Activity. Total degradation of chlorophyll without the inhibitor was resulted from both pheophorbide *a* and oxidative bleaching pathways. When 2 mM ascorbate was added, only the pheophorbide *a* pathway occurred because the ascorbate in the reaction mixture completely inhibited the oxidative bleaching pathway (Janave 1997). The contribution of the oxidative bleaching pathway to overall chlorophyll degradation was calculated by subtracting the absorbance change with inhibitor from the absorbance change without the inhibitor.

Experimental Design and Data Analysis. The experiment was a split-split plot design and replicated six times. The four sampling dates (3rd, 6th, 9th, and 12th) were the main plots within each trial. The three aphid treatments (control, *D. noxia*, and *R. padi*) were the subplots within each main plot (sampling dates), and the four wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*) were the sub-subplots within each subplot (aphid treatments). Six plants were sampled in each treatment on each sampling date (in total, 36 plants were used per treatment per sampling date) to measure chlorophyll degradation enzyme activities, and total protein contents in leaf samples. Data were analyzed using the PROC GLM procedure of the SAS software followed by TEST statements to ensure correct error terms used in assessing main effect of experimental factors (Cochran and Cox 1957, SAS Institute 1989). The means were separated using Fisher's least significant difference (LSD) test ($\alpha = 0.05$).

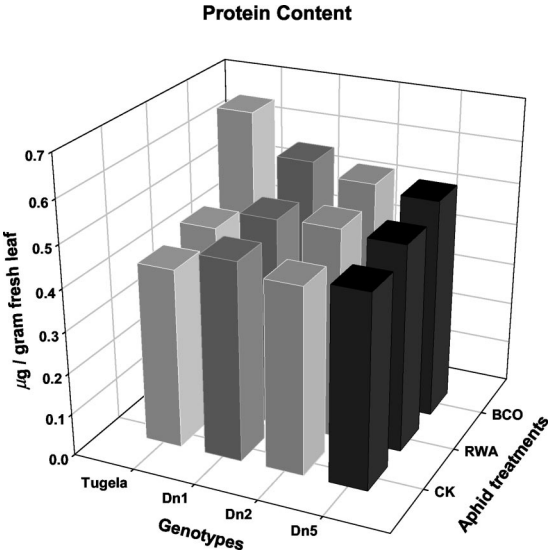

Fig. 2. Protein content in Tugela wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*). RWA, *D. noxia*; BCO, *R. padi*; CK, control.

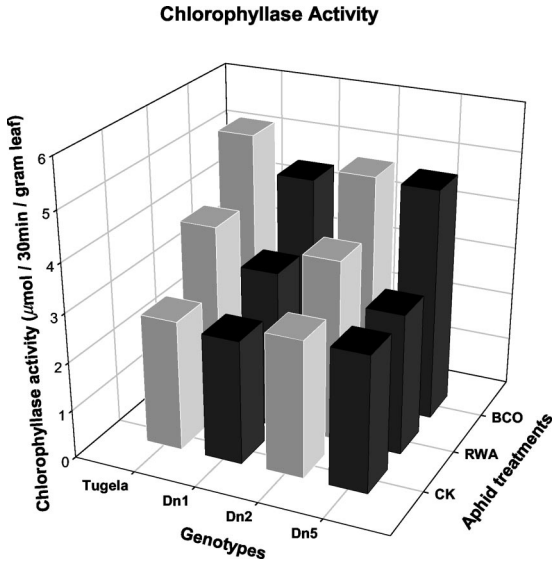

Fig. 3. Chlorophyllase activity in Tugela wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*). RWA, *D. noxia*; BCO, *R. padi*; CK, control.

Results

Total Protein Assay. There was no wheat line by aphid treatment by sampling date interaction observed on total protein concentration ($F = 0.57$; df = 18, 70; $P = 0.9125$). Only the aphid treatment by wheat line interaction significantly ($F = 2.66$; df = 6, 30; $P = 0.0343$) affected total protein concentration. Tugela plants infested with *R. padi* showed significantly higher protein content than other wheat lines with different aphid treatments (Fig. 2). None of the main effects (e.g., aphid treatment, sampling date, and wheat line) was significant ($P > 0.05$) on protein concentration.

Chlorophyllase Activity. Chlorophyllase activity among Tugela wheat lines was not significantly affected by the wheat line by aphid treatment by sampling date interaction ($F = 0.89$; df = 18, 65; $P = 0.5975$) or by the two-way interactions ($P > 0.05$). Chlorophyllase activities were significantly different among aphid treatments ($F = 10.13$; df = 2, 8; $P = 0.0064$) (Fig. 3), but not sampling dates ($F = 3.46$; df = 3, 11; $P = 0.0548$) or wheat lines ($F = 1.76$; df = 3, 12; $P = 0.2081$). Chlorophyllase activity was lower in *D. noxia*-infested plants than *R. padi*-infested plants (Fig. 3).

Mg-Dechelatase Activity. Mg-dechelatase activity was significantly affected by the wheat line by aphid treatment by sampling date interaction ($F = 2.53$; df = 18, 71; $P = 0.0029$). Therefore, analysis of Mg-dechelatase activity was conducted within each aphid treatment.

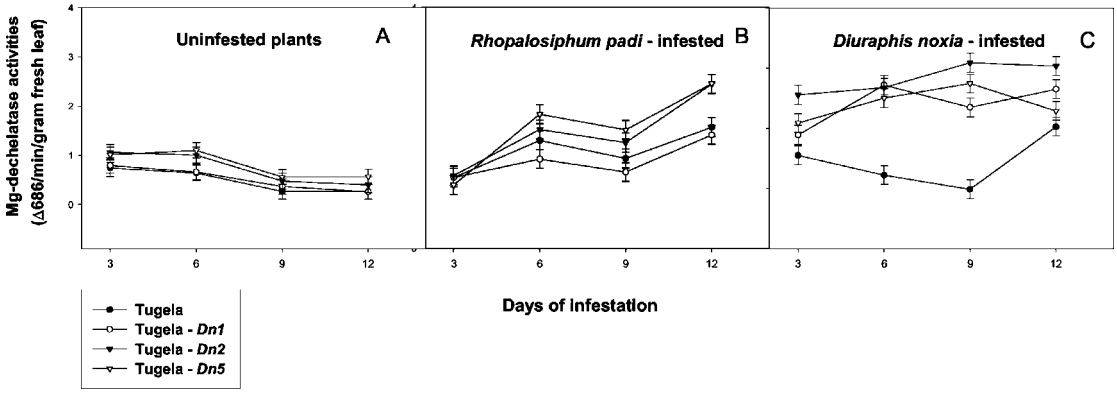

Fig. 4. Temporal changes of Mg-dechelatase activity in Tugela wheat lines infested with aphids. (A) Uninfested Tugela plants. (B) *R. padi*-infested Tugela plants. (C) *D. noxia*-infested Tugela plants. Each data point represents the mean (n = 6) on each sampling date. Error bar indicates the standard error of the mean.

Mg-dechelatase activity was lower in uninfested plants than in *R. padi*-infested and *D. noxia*-infested plants (Fig. 4). Wheat line by sampling date interaction was not significant on Mg-dechelatase activity among *R. padi*-infested plants ($F = 1.95$; df = 9, 36; $P = 0.0759$). Mg-dechelatase activity of *R. padi*-infested plants was significantly different among wheat lines ($F = 10.05$; df = 3, 12; $P = 0.0014$), but not among sampling dates ($F = 1.53$; df = 3, 12; $P = 0.2540$). Tugela-*Dn2* and *Dn5* had higher Mg-dechelatase activities than Tugela and Tugela-*Dn1* when infested with *R. padi* (Fig. 4B). Significant wheat line by sampling date interaction was observed among *D. noxia*-infested plants ($F = 2.67$; df = 9, 36; $P < 0.0173$). Mg-dechelatase activity was lower in *D. noxia*-infested Tugela compared with the other Tugela lines with *Dn* genes, except on day 12 that Mg-dechelatase activity was similar in Tugela and Tugela-*Dn5* plants (Fig. 4C).

Chlorophyll Oxidase Activity. Total degradation of chlorophyll was resulted from both pheophorbide *a* and oxidative bleaching pathways. Reaction with ascorbate resulted in only pheophorbide *a* pathway because the ascorbate inhibited the oxidative degradation of chlorophyll (Janave 1997). The chlorophyll oxidase activity was calculated by subtracting the absorbance change with ascorbate from the absorbance change without the ascorbate. All values calculated from this protocol were negative, which indicated no chlorophyll oxidative pathway involved in the enzymatic chlorophyll degradation.

Discussion

Wheat lines used in our experiment showed chlorophyll degradation enzyme (i.e., chlorophyllase and Mg-dechelatase) activities after being infested with both *D. noxia* and *R. padi.* However, chlorosis was observed only on *D. noxia*-infested plants. The chlorotic injury symptoms on *D. noxia*-infested Tugela wheat plants indicated the degradation of chlorophyll. Chlorophyllase and Mg-dechelatase play critical roles in the first two steps of the pheophorbide *a* pathway in chlorophyll catabolism (Matile et al. 1999, Dangl et al. 2000, Takamiya et al. 2000) (Fig. 1). The Tugela wheat lines were young seedlings (three-leaf stage) and still in an early stage of development. Unlike natural senescing plants, chlorophyll biosynthesis was, therefore, the main biological event occurring within the young green plant tissue.

Chlorophyllase activity was significantly higher in *R. padi*-infested wheat lines than *D. noxia*-infested plants (Fig. 3). Based on the chlorophyll degradation model (Fig. 1), more chlorophyllide *a* would be produced in the first step of chlorophyll degradation in *R. padi*-infested plants than *D. noxia*-infested plants. In contrast, Mg-dechelatase activity was significantly higher in *D. noxia*-infested plants than *R. padi*-infested plants (Fig. 4, B versus C). This means that higher concentrations of chlorophyllide *a* would have been degraded into pheophorbide *a* in *D. noxia*-infested plants when compared with *R. padi*-infested plants (Fig. 1). We hypothesize that in *R. padi*-infested Tugela wheat lines, Mg-chelatase and chlorophyll synthase activities were not affected. The excess concentration of chlorophyllide *a* produced by higher chlorophyllase activities would provide substrates for both chlorophyll catabolism (i.e., magnesium dechelation by Mg-dechelatase) and chlorophyll anabolism (i.e., chlorophyll formation by chlorophyll synthase). Thus, both chlorophyll degradation and chlorophyll biosynthesis were in balance in *R. padi*-infested wheat. Because *R. padi*-infested wheat had no observed chlorosis and lower Mg-dechelatase activity, chlorophyll biosynthesis catalyzed by chlorophyll synthase reused the chlorophyllide *a* into chlorophyll. In contrast, less chlorophyllide *a* was produced in *D. noxia*-infested Tugela lines because of lower chlorophyllase activity. Mg-dechelatase activity in *D. noxia*-infested plants was higher and caused a higher demand of chlorophyllide *a* that contributed to chlorophyll degradation. Chlorophyll synthase activity was most likely to be limited because there was a low level of chloro-

phyllide. These events would block chlorophyll *a* biosynthesis and contribute to chlorosis formation on *D. noxia*-infested wheat plants.

The four wheat lines used in this study had similar genetic backgrounds, except varying in one resistant gene. When under *D. noxia* infestation, Tugela plants showed more chlorotic injury symptoms than the Tugela isolines. However, Mg-dechelatase activity in Tugela was significantly lower than in Tugela *Dn* plants (Fig. 4C). We speculated that chlorophyll biosynthesis enzyme (i.e., Mg-chelatase and chlorophyll synthase) activities in *D. noxia*-infested plants are less affected in Tugela isolines with *D. noxia*-resistant *Dn* genes but strongly inhibited in Tugela plants (Fig. 1). Both chlorophyll biosynthesis and degradation occurred in Tugela *Dn* plants and thus would decrease the formation of chlorotic symptoms. Inhibition of chlorophyll biosynthesis enzymes (i.e., Mg-chelatase and chlorophyll synthase) in *D. noxia*-susceptible Tugela plants would limit the capacity of chlorophyll biosynthesis and would account for severe chlorosis.

As reported previously by Ni et al. (2001, 2002), chlorotic symptoms on *D. noxia*-infested wheat leaves were most likely to be correlated with Mg-dechelatase activities. We detected higher chlorophyllase and lower Mg-dechelatase activity in *R. padi*-infested Tugela wheat lines compared with *D. noxia*-infested plants (Figs. 3 and 4). Additionally, among *D. noxia*-infested wheat lines, we detected higher Mg-dechelatase activity in Tugela lines with *Dn* genes than *D. noxia*-susceptible Tugela (Fig. 4C). No chlorophyll oxidase activity was detected from any of the leaf samples. Chlorosis formation elicited by *D. noxia* feeding was, therefore, most likely to be related to the activities of these two degradative enzymes.

Acknowledgments

We thank Y. Liu and P. Hofer for assistance on conducting experiments and maintaining the plant and aphid colonies and S. Blodgett and P. Bruckner for critical review of the manuscript. We also thank James F. Robison-Cox and John J. Borkowski for advice on data analysis. The research reported here was supported in part by the USDA–NRICGP 99-35302–8628. This is journal series no. 2003-26 from the Montana Agricultural Experiment Station.

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Received 3 August 2003; accepted 19 December 2003.